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(3)

(19) (A) # CANADIAN PATENT

(54) PROCESS FOR PREPARING ESTERS OF 6-DEOXY-5-OXYTETRACYCLIN

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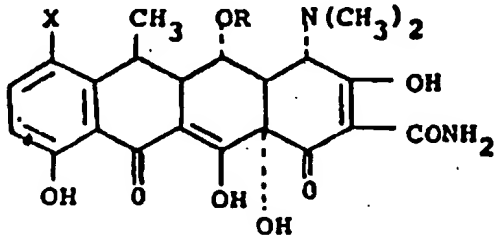
No. OF CLAIMS 14 - No drawing

Object of th pr sent invention is a process for preparing esters of 6-deoxy-5-oxytetracycline, the addition salts thereof with non-toxic pharmaceutically acceptable acids. More particularly, object of the present invention is a process for preparing 6-deoxy-5-oxytetracycline-5-esters of the structure



III

wherein R is a radical of a mono- or dicarboxylic organic acid having from 1 to 10 carbon atoms and X is hydrogen, chlorine and bromine, the addition salts thereof with non-toxic pharmaceutically acceptable acids. The compounds of general formula III as herein defined, of which those in which R stands for a formyl radical or a radical of a halosubstituted monocarboxylic acid or of a dicarboxylic acid, each having from 1 to 10 carbon atoms in the molecule are novel,and are included within the scope of the invention. Said esters have proved to possess a good antibiotic activity. Some are characterized by a very good adsorption after oral administration, giving blood levels higher than those of the corresponding 6-deoxy-5-oxytetracycline non-acylated in position 5. Others are characterized by a good antibiotic activity also on tetracycline-resistant strains.

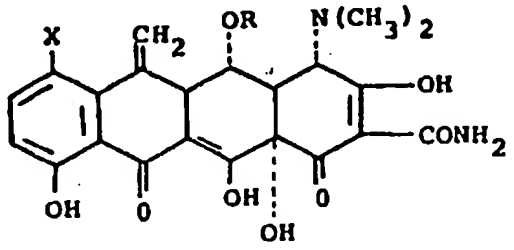
It is known from the literature (J.Am.Chem.Soc. 84, 2645,1962) that two different steric configurations of the methyl group exist in position 6 of the tetracycline skeleton, usually indication as "epi" and"normal", better as "α" and "β".

The configuration of these tetracyclines and of their esters in position 5 can be easily determined by means of the N.M.R. spectroscopy.

In fact, the 6α-derivatives show, in dimethylsulphoxide, the signal (dupl t) due to CH_3 at about 1,5δ while the 6β

derivativ s show the same signal at about 1σ.

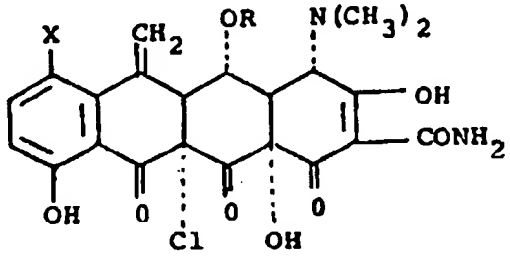
In the Canadian Patent No. 931,946 filed on August 5, 1970 of the Applicant, are described and claimed new tetracycline derivatives and namely 6-demethyl-6-deoxy-6-methylene-5-acyloxy-tetracyclines of the structure



I

wherein R is a radical of an organic acid containing from 1 to 10 carbon atoms and X is hydrogen, chlorine and bromine.

In the above Canadian patent application are also described and characterized new 11α-chloro-6-demethyl-6-deoxy-6-methylene-5-acyloxy-tetracyclines of the structure:



II

wherein R and X have the above meaning.

Said compounds are intermediates useful not only for preparing the derivatives I according to the above patent application, but also the derivatives III of the present patent application as it will be further detailed.

Now it has been found, and this is the object of the present invention, that the reduction of a compound selected from the group of 6-demethyl-6-deoxy-6-methylene-5-formyloxytetracyclines (V) and of 11α-chloro-6-demethyl-6-deoxy-6-methylene-5-acyloxy-

tetracyclines (II) with hydrogen in the presence of a suitable catalyst of the platinum group, supplies a mixture of α-6-deoxy-5-acyloxytetracyclines and β-6-deoxy-5-acyloxytetracyclines (III) which can be separated into the two epimers by means of suitable chemical-physical methods known per se in the literature, as countercurrent distribution, column chromatography or fractionated precipitation.

Alternately α-6-deoxy-5-acyloxytetracyclines and β-6-deoxy-5-acyloxytetracyclines (III) can be prepared by direct acylation of the corresponding compound having the hydroxyl-group in 5 free (III, R = H).

Said acylation is carried out, for example, with the desired organic acid in the presence of a strong acid selected from the group consisting of hydrofluoric, methanesulphonic and ethane-sulphonic acid.

According to the process of the present invention, the 6-demethyl-6-deoxy-6-methylene-5-formyloxytetracyclines of structure I are dissolved or suspended in a suitable solvent and reacted with hydrogen at a suitable temperature and under suitable pressure in the presence of catalytic quantities of a metal of the platinum group.

The quantity of hydrogen adsorbed is regulated so as to reduce the methylene group in 6 to methyl group, and other reducible groups such as 11α-chloro- and 7-halo-group, when present in the molecule.

The solvent or suspension medium, in which the catalytic reduction is carried out, is selected so that it does not interfere with the catalyst or with the starting-or end-tetracycline compound. Polar organic solvents are suitable for this purpose and consist of lower aliphatic alcohols such as methanol,

thanol, water-miscible ethers such as tetrahydrofuran and di xane, lower aliphatic acids such as anhydrous or aqueous f rmic and acetic acids. It is sometimes preferable to perform the reduction in the pr sence of small quantities of strong mineral acids such as hydrochloric acid.

As to the temperature conditions and to the hydrogenation pressure, there are no critical values to be observed. The operation is generally performed at from 0° to 50°C and preferably at room temperature and between 100 atmospheres and room pressure.

Platinum, palladium, rhenium, rhodium, ruthenium and iridium and their oxides and chlorides are employed as catalyst of the platinum group. The catalyst can be dispersed as such or deposited on suitable supports as charcoal, silica or barium sulphate. Once the reduction is over, the catalyst is filtered, and the reaction product is isolated and purified according to the usual techniques in the tetracyclines field.

The reduction product consists, as above mentioned, of a mixture in variable ratios of the two epimers, which is preferably separated to point out the different pharmacological properties of the epimers themselves. Said separation is performed employing the methods known per se in the tetracycline field, such as fractionated precipitation, countercurrent distribution and column chromatography.

The diphase solvents mixtures employed, usually consist of buffer solutions at a pH ranging from 2 to 6, for example McElvain buffer and of water-immiscible solvents selected from the group of normal and "iso" butyl and amyl-alcohols, of mixed ketones, such as methylisobutylketone, and of chlorinated solvents from the countercurrent distribution device come,

first, the least polar fractions and later, little by little, those having an increasing polarity. Also the column chromatography can be advantageously employed with a suitable support and with a suitable elution system.

Good results are obtained using cellulose and a solvents mixture similar to that reported above for the countercurrent. Owing to the anphoteric nature, the compounds of the present invention can be employed in therapy as such or transformed into their salts with non-toxic pharmaceutically acceptable acids. Complexes with calcium have been also prepared. Alternately α-6-deoxy-5-acyloxytetracyclines and β-6-deoxy-5-acyloxytetracyclines can be prepared by direct acylation of the corresponding 6-deoxy-5-oxytetracyclines. Said acylation can be performed treating 6-deoxy-5-oxytetracycline with a mono- or dicarboxyclic organic acid having from 1 to 10 carbon atoms in the presence of a strong acid selected from the group consisting of hydrofluoric, methanesulfonic and ethanesulfonic acid.

Also in this case, in order to isolate and purify the acylated product in 5, the same methods described to isolate and purify the same compounds prepared by catalytic reduction of the corresponding 6-methylene-derivatives can be followed.

The compounds of the invention are particularly interesting as anti-bacteric products: some possess an antibacteric activity on tetracycline-resistant strains, others have a very good oral adsorption, giving blood levels much higher than those given by doxycycline or α-6-deoxy-5-oxytetracycline, with which the compounds of the present invention have a close structural analogy. The activity on tetracyclin -resistant strains has been tested "in vitro" in comparison with that of doxycycline.

Table 1 r ports the values of MIC r minimum inhibiting concentration, expressed in µg/ml which is the minimum quantity of substance capable of inhibiting completely in vitro the development of the microorganism under examination.

TABLE 1

Activity on tetracycline-resistant Staphylococcus aureus ATCC 12715

Compound	MIC (in µg/ml)
α-6-deoxy-5-oxytetracycline (doxycycline)	20.0
α-6-deoxy-5-acetyloxytetracycline	5.0
β-6-deoxy-5-acetyloxytetracycline	20.0
α-6-deoxy-5-(2-ethyl-butyryl)-oxytetracycline	0.3
β-6-deoxy-5-(2-ethyl-butyryl)-oxytetracycline	0.6
α-6-deoxy-5-(β-chloropropionyl)-oxytetracycline	5.0

In Table 2 there are reported some results of a comparison test "in vivo" between doxycycline and the first member of the compounds of the present invention, that is α-6-deoxy-5-formyl-oxytetracycline.

This comparison test has been carried out in the mouse (12 animals per group) infected intraperitoneously with Staphylococcus aureus PV and the two antibiotics under examination have been administered orally 4-24-48-72 hours after the infection. The symbol PD_{50} means Protective Dose 50.

TABLE 2

Compound	dose mg/kg	mortality percentage on the 11th day	PD_{50}
Control	-	100	
Doxycycline	50	0	
	25	16	15
	12.5	58	
	6.25	100	
α-6-deoxy-5-formyl-oxytetra-cycline	50	0	
	25	0	7.5
	12.5	16	
	6.25	58	

From Table 2 it is evident that the compound of the present invention shows a PD_{50} which is the half of the corresponding value of doxycycline.

In Table 3 are reported some results of another comparison test in the mouse (12 animals per group) infected intraperitoneously with Salmonella abortivo equina (Gram-negative-strain). Also in this test the two antibiotics under examination have been administered orally 4-24-48-72 hours after the infection.

TABLE 3

Compound	dose mg/kg	mortality percentage	PD_{50}
Control	-	100	
Doxycycline	50	25	40
	25	75	
α-6-deoxy-5-formyloxy-tetracycline	50	0	25
	25	50	

In Table 4 are reported the comparison data of the acut toxicity (LD_{50} in g/kg body weight) in the mouse "per os" (groups of 10 animals per dose) between doxycycline and α-6-deoxy-5-formyloxytetracycline.

TABLE 4

Compound	LD_{50}	(fiduciary limits)
doxycycline	2.140	2.09-2.20
α-6-deoxy-5-formyl-oxy-tetracycline	4.68	3.92-5.57

The following examples are to illustrate the invention without limiting it.

EXAMPLE 1

6-Deoxy-5-formyl-oxytetracycline

1.5 Grams of 6-deoxy-6-demethyl-6-methylene-5-formyloxy-tetracycline are dissolved in 30 ml of formic acid and hydrogenated under atmospheric pressure and at room temperature with 500 mg of PtO_2.

After about an hour and 20 minutes, the reaction is over (disappearance of the maximum at λ = 240 mμ in the U.V. spectrum). The product is filtered, the formic acid is evaporated off below 35°C in "vacuo" and taken up with ethyl acetate-methanol 1 : 1 decolouring with charcoal.

After concentration in "vacuo" the product is precipitated with ethyl ether. By adding petroleum ether to the mother liquors, some further product is recovered for an amount of 900 mg. Countercurrent distribution with a solvents mixture consisting of 1100 ml of Mc Elvain buffer (pH 4,6), 480 ml of methylisobutylketone, 480 ml of ethyl acetate and 210 ml of butanol,

giv s α-6-deoxy-5-formyl-oxytetracycline and β-6-deoxy-5-formyloxytetracycline showing a duplet at δ 1.02 at the NMR spectrum (DMSO-d_6-$CDCl_3$ 1 : 1). At the UV, α-6-deoxy-5-formyloxy-tetracycline shows a maximum at 268 mμ and another maximum at 345 mμ in a solution consisting of 80 parts of 0,01N aqueous hydrochloric acid and 20 parts of methyl alcohol. At the I.R. (in KBr) it shows the maxima at 1730 cm^{-1} and 1165 cm^{-1}.

The "Nuclear Magnetic Resonance" spectrum in $CDCl_3$ shows the following characteristic signals : δ1,49 (d, 3H); δ2,60 (s, 6H); δ5,95 (m, 1H); δ6,7-7,7 (m, 3H); δ7,95 (s, 1H). The product shows the following minimum inhibiting concentrations (between brackets is reported the corresponding value of doxycycline) : E coli B : 0,3 (0,62); Klebsiella pneumoniae 0,15 (0,62); Mycobacterium sp. ATCC 607 0,07 (0,15); see also tables 2 and 3.

EXAMPLE 2

6-Deoxy-5-formyl-oxytetracycline

a) Preparation of the intermediate 11α-chloro-6-demethyl-6-deoxy-6-methylene-5-formyloxy-tetracycline.

20 g of 11α-chloro-5-hydroxy-tetracycline 6,12-hemiketale are suspended in 100 ml of 99% formic acid in a polythene flask. The product is cooled externally with a refrigerating mixture at -15°C and 100 ml of anhydrous hydrofluoric acid are added. The temperature is allowed to rise to the room value. Then the reaction mass is allowed to stand at this temperature for about 20 hours.

A nitrogen stream is passed to remove the hydrofluoric acid, the mixture is evaporat d in "vacuo" to little volume, and the residue is poured into about 2 liters of ethyl ether. It is stirred for 10 minutes and then filtered. The product obtained

is washed on a filter with ethyl ether, dried in "vacuo", and recrystallized from methanol-ether. A 3 l autoclave is charged with the recrystallized product obtained as above described, then are added 500 ml of methanol and 5 g of catalyst consisting of 5% rhodium on charcoal. The pressure of hydrogen is adjusted to between 3 and 5 atmospheres and the product is kept with stirring at room temperature for 12 hours.

Further 5g of catalyst are added and the whole is hydrogenated for 12 hours. It is filtered and the filtration cake is washed with methanol and the filtrate and the combined washings are evaporated in "vacuo" to dryness. The solid residue is pulped with little ether and filtered. The crude product thus obtained is dissolved in a buffer solution at pH 4,5. Separately there is prepared a chromatographic column (h = 150 cm; ø 3 cm) filled with cellulose pulped in the same buffer solution as above, saturated with ethyl acetate and methyl-isobutylketone 1 : 1. The solution of the product is chromatographed through this column eluting with ethyl acetate/methyl-isobutylketone (1 : 1) saturated with a buffer solution at pH 4,5.

In the first fraction α-6-deoxy-5-formyloxytetracycline is collected, in the second fraction passes a small quantity of 6-demethyl-6-deoxy-6-methylene-5-formyloxy-tetracycline; the third fraction contains β-6-deoxy-5-formyloxytetracycline.

EXAMPLE 3

6-Deoxy-5-acetyloxytetracycline

3 Grams of 11α-chloro-6-deoxy-6-demethyl-6-methylene-5-acetyloxy-tetracycline are dissolved in 50 ml of a mixture water-ethanol 1:1 with 3 ml of aqueous concentrated hydrochloric acid. 1 g of charcoal palladium is added and the product is

hydrogenated at room temperature under 5 atmospheres, following the reduction process at the U.V. At the end of the operation, the whole is filtered and evaporated in "vacuo" to dryness.

The residue is taken up with methanol and decoloured with charcoal. After concentration to little volume, the product is precipitated by dilution with three volumes of ethyl ether. The crystalline product consisting of the α-6-deoxy-5-acetyloxy-tetracycline hydrochloride mixture, is subjected to countercurrent distribution as described in Example 1. The former fractions consist of the α-6 epimer and the latters consist of the β-6 epimer.

At the U.V., α-6-deoxy-5-acetyloxytetracycline shows two maxima at 272 and 345 mμ (in a solution of diluted hydrochloric acid and methanol). At the I.R. (in KBr) it shows maxima at 1745 cm^{-1} and at 1240 cm^{-1} and at 1240 cm^{-1}.

At the N.M.R. in $CDCl_3$ it shows the following characteristic signals:

δ1,45 (d, 3H); δ2,10 (s,3H); δ2,56 (s,2H); δ5,67 (m, 1H); δ6,75-7,6 (m, 3H).

At the N.M.R. in $CDCl_3$, β-6-deoxy-5-acetyloxytetracycline shows the following characteristic signals:

δ1,01 (d, 3H); δ2,19 (s, 3H); δ2,46 (s, 6H); δ5,25 (two d, 1H); δ6,5-7,6 (m, 3H).

Besides the MIC values (in μg/ml) reported in Table 1, β-6-deoxy-5-acetyloxytetracycline shows a MIC of 0,03 (doxycycline 0,15), in comparison with the strain Mycobacterium sp.ATCC 607.

EXAMPLE 4.

6-Deoxy-5-(2-ethylbutyryl)-oxytetracycline

1,0 Gram of 11α-chloro-6-deoxy-6-demethyl-6-methylene-5-(2-ethyl-butyryl)-oxytetracycline is dissolved in 30 ml of a

mixture water/ethanol 1:1 with 1 ml of concentrated hydrochloric acid.

300 mg of Adams catalyst (PtO_2/C) are added and the product obtained is hydrogenated at room temperature and at 5 atmospheres. The reaction course is followed at the U.V. (the max disappears at λ = 239 mμ). When the reaction is over, the whole is filtered to remove the catalyst, evaporated in "vacuo" to dryness and the product is purified by countercurrent according to the process described in Example 3.

At the U.V. α-6-deoxy-5-(2-ethylbutyryl)-oxytetracycline shows two maxima at 272 and 345 mμ. At the I.R. it shows the maxima at 1735 and 1235 cm^{-1}.

At the N.M.R. in $CDCl_3$ it shows the following characteristic signals:

δ0,91 (t, 6H); δ1,3-1,8 (m, 7H); δ 2,57 (s, 6H); δ 5,80 (m, 1H); δ6,75-7,65 (m, 3H).

EXAMPLE 5

6-Deoxy-5-formyl-oxytetracycline

1,0 Gram of α-6-deoxy-5-oxytetracycline hydrochloride and 10 ml of formic acid are dissolved in 30 ml of anhydrous hydrofluoric acid.

The mixture is allowed to stand at room temperature for 115 hours, then the hydrofluoric acid is removed by a nitrogen stream. The residue is poured into 300 ml of ethyl ether in which it precipitates as yellow solid. It is filtered and the solid is dissolved in the lower phase of the solvents mixture prepared with 1100 ml of McElvain buffer (pH 4,6), 480 ml of methylisobutylketone, 480 ml of ethyl acetate and 200 ml of butylic alcohol.

The minimum quantity of the lower phase sufficient to

dissolve the product is employed, and the latter is simultaneously released as base and then extracted three times with the upper phase of the above solvents mixture.

The upper phase is washed ten times with the lower phase and then it is dried. The residue is dissolved in 50 ml of ethyl acetate, washed twice with distilled water, dried over anhydrous sodium sulphate, decoloured with charcoal and crystallized from ethyl acetate-petroleum ether. α-6-Deoxy-5-formyloxy-tetracycline is obtained. Its characteristics are reported in Example 1. Analogously, starting from β-6-deoxy-5-hydroxytetracycline is obtained β-6-deoxy-5-formyloxytetracycline.

At the N.M.R. (DMSO-d_6 -$CDCl_3$ 1 : 1) it shows a characteristic duplet at 1.02.

EXAMPLE 6

6-Deoxy-5-acetyloxytetracycline

1,0 Gram of α-6-deoxy-5-oxytetracycline hydrochloride and 10 ml of glacial acetic acid are dissolved in 30 ml of anhydrous hydrofluoric acid and allowed to stand at room temperature for 115 hours. Hydrofluoric acid is removed with a nitrogen stream and the residue is poured into 300 ml of ethyl ether. The hydrochloride precipitated from the ethyl ether and separated by filtration, is dissolved in the lower phase of the solvents mixture already described in Example 5 in order to release the base which is extracted three times with the upper phase.

The latter is evaporated to dryness, the residue is taken up with 50 ml of ethyl acetate and washed twice with distilled water. The product obtained is dried over sodium sulphate, decoloured with charcoal and crystallized from ethyl acetate and petroleum ether : α-6-deoxy-5-acetyloxytetracycline is obtained, having the characteristics reported in Example 3.

EXAMPLE 7

6-Deoxy-5-(β-chloropropionyl)-oxytetracycline

Operating as in Example 5, but employing β-chloropropionic acid instead of formic acid, α-6-deoxy-5-(β-chloropropionyl)-oxytetracycline is obtained. At the U.V. α-6-deoxy-5-(β-chloropropionyl)-oxytetracycline shows two maxima at 272 and 345 mμ.

At the I.R. (in KBr) it shows maxima at 1740 and 1240 cm^{-1}. At the N.M.R. (in $CDCl_3$) it shows the following characteristics signals:

δ1,47 (d, 3H); δ2,56 (s, 6H); δ2,82 (t, 2H); δ3,79 (t,2H); δ5,78 (m, 1H); δ6,7-7,6 (m, 3H).

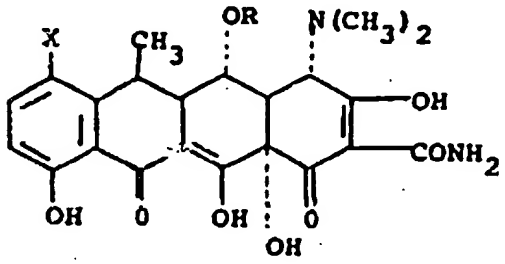
EXAMPLE 8

α-6-Deoxy-5-succinyloxytetracycline

Operating as in the previous Example 7, but employing succinic acid instead of acetic acid, the corresponding ester is obtained.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the preparation of 6-deoxy-5-oxytetracycline-5-esters and its addition salts with non-toxic pharmaceutically acceptable acids of the following structure:

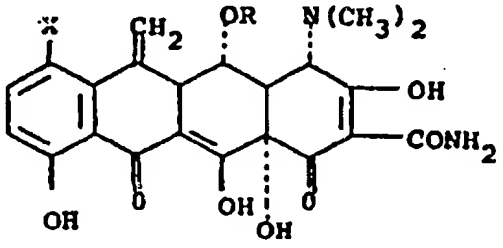


Formula I

wherein R is a formyl radical or a radical of a halosubstituted mono- or dicarboxylic acid each having from 1 to 10 carbon atoms in the molecule and X is selected from the group consisting of hydrogen, chlorine and bromine, comprising:

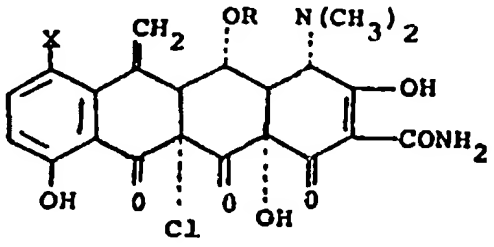
(i) reacting a compound selected from the group consisting of

Formula II



and

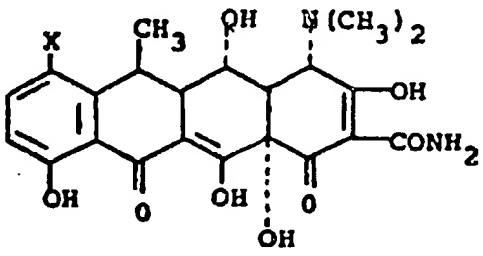
Formula IIa



Claim 1 continued:

and th ir addition salts, wherein R and X have the above m aning, with hydrogen in the presence of a suitable catalyst of the platinum group at a temperature of from 0° and 50°C and between the atmospheric pressure and 100 atmospheres, until 1 to 3 moles of hydrogen per mole of tetracycline compound are absorbed and in that the mixture of α-6 or β-6 epimer thus formed is isolated and purified as such or separated into its components by the usual techniques,

or (ii) reacting a compound of the following structure:



Formula IV

and their addition salts wherein X has the above meaning, with formic acid or a halosubstituted mono- or dicarboxylic organic acid having from 1 to 10 carbon atoms in the presence of a strong acid selected from the group consisting of hydrofluoric, methanesulphonic and ethanesulphonic acid and in that the product thus formed is isolated and purified in a manner known "per se".

2. The process according to claim 1 characterized in that the catalyst consists of PtO_2.

3. The process according to claim 1 characterized in that the catalyst consists of rhodium.

D

4. The process according to claim 1 characterized in that the catalyst consists of palladium.

5. The process according to claim 1 characterized in that th esterification is carried out in the presence of hydrofluoric acid.

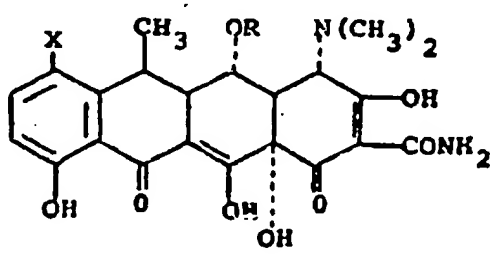
6. The process as claimed in claim 1 in which 6-deoxy-6-demethyl-6-methylene-5-formyloxytetracycline was hydrogenated in the presence of PtO_2.

7. The process as claimed in claim 1 in which 6-deoxy-6-demethyl-6-methylene-5-formyloxytetracycline was hydrogenated in the presence of Adams catalyst (PtO_2/C).

8. The process as claimed in claim 1 in which 11α -chloro-6-deoxy-6-demethyl-6-methylene-5-formyloxytetracycline was hydrogenated in the presence of charcoal palladium.

9. The process as claimed in paragraph (ii) of claim 1 in which β -6-deoxy-5-oxytetracycline hydrochloride is reacted with α-chloropropionic acid in the presence of hydrofluoric acid.

10. Esters of 6-deoxy-5-oxytetracycline and of if its addition salts with non-toxic pharmaceutically acceptable acids having the structure



Formula I

Claim 10 continued:
wherein R is a formyl radical or a radical of a halosubstituted mono- or dicarboxylic acid each having from 1 to 10 carbon atoms in the molecule and X is selected from the group consisting of hydrogen, chlorine and bromine, whenever prepared by the process of claim 1.

11. 6-Deoxy-5-formyloxytetracycline whenever prepared by the process of claim 6.

12. α-6-Deoxy-5-formyloxytetracycline whenever prepared by the process of claim 7.

13. β-6-Deoxy-5-formyloxytetracycline whenever prepared by the process of claim 8.

14. β-6-Deoxy-5-(β-chloropropionyl)-oxytetracycline whenever prepared by the process of claim 9.

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